Aerobic degradation of 2,4-DB in aquatic systems

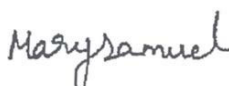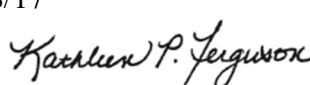|	
Report:	MRID 49975801. Clark, B. 2016. [^{14}C]2,4-DB: Aerobic Aquatic Metabolism in Two Water-Sediment Systems. Unpublished study performed by ABC Laboratories, Inc., Columbia, Missouri; sponsored and submitted by 2,4-DB Task Force, c/o Data Group Management, Raleigh, North Carolina. ABC Study No. 82630. Test systems were treated on December 7, 2015 (Goose River) and January 11, 2016 (Chula; p. 21); a termination date was not reported. Final report issued July 20, 2016.
Document No.:	MRID 49975801
Guideline:	OPPTS 835.4300
Statements:	The study was conducted according to USEPA FIFRA GLP standards (p. 3). Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). The authenticity of the report is certified in the Quality Assurance statement (p. 4).
Classification:	This study is classified as supplemental. The study failed to use solvents with a range of dielectric constants (including a nonpolar solvent) to maximize extraction of the residues; unextracted residues totaled up to 28.9% of the applied. In the Goose River water:clay loam sediment system, overall recoveries were below guideline criteria (<90%) in multiple samples beginning at 17 days, and in the Chula system, material losses of >10% occurred in multiple samples. Additionally, there was inter-replicate data variability.
PC Code:	030801
Final EPA Reviewer:	Cheryl Sutton, Ph.D. Environmental Scientist	Signature: CHERYL SUTTON *(Digitally signed by CHERYL SUTTON Date: 2018.03.28 09:12:29 -04'00')* Date: 03/28/2018
CDM/CSS-Dynamac JV Reviewers:	Mary Samuel, Environmental Scientist	Signature: *Mary Samuel* Date: 3/28/17
	Kathleen Ferguson, Ph.D., Environmental Scientist	Signature: *Kathleen P. Ferguson* Date: 3/28/17

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel.

Executive Summary

The aerobic transformation of [phenyl-U-^{14}C]-labeled 2,4-DB was studied in a water:clay loam sediment system (Goose River; water pH 8.0, sediment pH 7.5 and organic carbon 4.5%) from North Dakota and a water:sand sediment system (Chula; water pH 8.3, sediment pH 5.7 and organic carbon 0.49%) from Georgia in closed systems treated at 0.936-1.030 µg a.i./mL and incubated in the dark at 20°C for 50 days. Duplicate samples (two entire vessels) of each test system were collected at each sampling interval. In the water column of the Goose River water:clay loam sediment systems following treatment, measured redox potentials, oxygen saturation and pH were +260 to +393 mV, 7.50-8.53 mg/L and 8.155-8.651, respectively, with measured redox potentials

and pH in the sediment of -148 to -81 mV and 6.449-7.266, respectively. In the water column of the Chula water:sand sediment systems following treatment, measured redox potentials, oxygen saturation and pH were +212 to +448 mV, 4.69-8.49 mg/L and 4.440-8.267, respectively, with measured redox potentials and pH in the sediment of -159 to -76 mV and 5.016-7.560, respectively. The pH of the water and sediment decreased steadily beginning at 14-17 days. In both test systems, the water was oxic and the sediment was suboxic during the experiment. Both test systems were viable throughout the experiment.

In the Goose River water:clay loam sediment system, overall recoveries averaged $94.9 \pm 6.9\%$ (sample range 82.5-102.2%) of the applied. Recoveries generally were near or below guideline criteria (<90%) beginning at 17 days. In the water columns, 2,4-DB was a maximum of 95.1% of the applied at time 0 and decreased to $\leq 0.1\%$ at and after 17 days posttreatment. In the sediment, 2,4-DB was a maximum of 21.1% at 14 days and decreased to 2.1% at 50 days.

In the Chula water:sand sediment system, overall recoveries averaged $98.6 \pm 5.2\%$ (sample range 92.2-107.0%) of the applied. Recoveries were within guideline criteria (90-110%). In the water columns, 2,4-DB was a maximum of 97.4% of the applied at time 0, decreased to 2.0% at 28 days posttreatment and was 0.0% at 50 days. In the sediment, 2,4-DB was a maximum of 13.0% at 7 days and decreased to 2.2% at 50 days.

Observed DT_{50} values, calculated half-lives, and information on transformation products are listed in **Table 1**. 2,4-DB dissipated with SFO DT50 values of 9.15 days in the Goose River water:clay loam sediment system and 9.28 days in the Chula water:sand sediment system. Product profiles were comparable between the two systems. 2,4-D was the only nonvolatile transformation product identified.

In the Goose River water, total radioactive residues decreased from 97.3% of the applied at time 0 to 1.6% at 50 days posttreatment. In the sediment, extractable radioactivity increased from 4.4% at time 0 to a maximum of 26.3% at 14 days and was 4.2% at 50 days. Unextracted radioactivity increased from 0.8% at time 0 to a maximum of 28.9% at 28 days and was 18.7% at 50 days. CO_2 was a maximum of 77.1% at 50 days. Organic volatiles were <LOD at study termination.

In the Chula water, total radioactive residues decreased from 98.7% of the applied at time 0 to 2.9% at 50 days posttreatment. In the sediment, extractable radioactivity increased from 4.8% at time 0 to a maximum of 15.1% at 14 days and was 4.8% at 50 days. Unextracted radioactivity increased from 1.8% at time 0 to a maximum of 21.1% at 21 days and was 15.2% at 50 days. CO_2 was a maximum of 71.0% at 50 days. Organic volatiles were <LOD at study termination.

Table 1. Results Synopsis: Aerobic Aquatic Metabolism of 2,4-DB in the Total System[1]

Total System	Observed DT_{50} (days)	Calculated Half-life (days)[1]	Model Parameters and Statistics[1]	Transformation Products Common Name (maximum % AR, associated interval)[2]	
				Major	Minor
North Dakota USA Goose River water:clay loam sediment (20°C, water pH 8.0, sediment pH 7.5)	14-17	9.15 SFO	$C_0 = 109$ $k = 0.0757$ $S_C = 1.82e+03$ $S_{SFO} = 2.01e+03$	Unextracted residues (28.9%, 28 days) CO_2 (77.1%; 50 days)	2,4-D (7.8%, 14 days)
Georgia, USA Chula water: sand sediment (20°C, water pH 8.3, sediment pH 5.7)	14-17	9.28 SFO	$C_0 = 115$ $k = 0.0747$ $S_C = 3.53e+03$ $S_{SFO} = 3.72e+03$	2,4-D (14.3%, 14 days; observed DT50 <3 days) Unextracted residues (21.1%, 21days) CO_2 (71.0%; 50 days)	None

1 Calculated half-lives and model parameters in accordance with NAFTA kinetics guidance (USEPA, 2012); Single First Order (SFO).
2 AR means "applied radioactivity".
Trapped CO_2 (as carbonate) was recovered from the water at a maximum 9.0-9.6% of the applied at 17 days from the two test systems.

I. Materials And Methods

A. Materials:

1. Test Material   [Phenyl-U-^{14}C]-labeled 2,4-DB (p. 17)
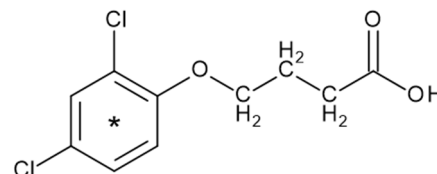
    Specific activity: 49.14 µCi/mMol

    Radiochemical purity: 97.8%

    Chemical purity: Not reported

    Lot No.:   9040AKV001-3

Solubility in water: Not reported

2. Reference Compounds: The following compounds were used in the analysis.

Table 2. Reference Compounds.

Applicant's Code Name	Chemical Name	Purity (%)	Lot No.
2,4-DB	4-(2,4-Dichlorophenoxy)butyric acid	99.93	RPH/566/95
4-CPAA	4-Chlorophenoxyacetic acid	99.86	S1137
2,4-D Technical Acid	(2,4-Dichlorophenoxy)acetic acid	99.98	SP547-55

Data obtained from p. 18 and Figure 1, p. 52, of the study report.

3. Water:Sediment: Water and sediment collection and characterization are summarized in **Table 3** and **Tables 4a-4b**, respectively.

Table 3. Water:Sediment Collection and Storage.

Description		Goose River	Chula
Geographic location		Grand Forks, North Dakota	Chula, Georgia
Site description		Not reported	
Pesticide use history at the collection site		Not reported. No fertilizers used for the past 4 years.	
Collection date		November 19, 2015	November 23, 2015
Collection procedures	Water:	Not reported	
	Sediment:	Not reported	
Storage conditions	Water:	Sediment stored with overlying water at *ca.* 4°C in the dark.	
	Sediment:		
Storage length		≤1 month	
Preparation	Water:	Sieved (*ca.* 2-mm mesh)	
	Sediment:		

Data obtained from p. 191 in the study report.

Table 4a. Parameters for Characterization of Water:Sediment Samples – Goose River.

Parameter (unit)	Field Sampling/Post Handling	Stage of Test Procedure		
		0 Days	28 Days	50 Days
Water				
Temperature (ºC)	--			
pH	8.0	8.538	8.199	8.287
Hardness (mg/L as CaCO$_3$)	716			

Parameter (unit)	Field Sampling/Post Handling	Stage of Test Procedure		
		0 Days	28 Days	50 Days
TOC (ppm)	9.4	11.1		10.3
O_2 Content (mg/L)	--	8.34	7.93	8.53
Redox potential (mV)		+283	+335	+393
Sediment				
Sampling Depth	--			
pH[1]	7.5	6.951	6.513	7.266
Soil Texture (USDA)	Clay loam			
Particle Size Distribution (%) — Sand	28			
Silt	40			
Clay	32			
Organic matter (%) (Furnace Method)	7.0			
Organic carbon (%) (Walkley Black)	4.5			
CEC (meq/100 g)	23.7			
Microbial biomass (µg C/g sediment)	--	467.0		574.3
Redox potential (mV)		-148	-105	-118

Data obtained from p. 31; Table 1, pp. 38-39; and Table 4, p. 43, in the study report. USDA sediment texture confirmed using USDA-NRCS technical support tools.
1 Post-handling pH of sediment determined as 1:2, sediment:0.01M $CaCl_2$.
-- = not reported.

Table 4b. Parameters for Characterization of Water:Sediment Samples – Chula.

Parameter (unit)	Field Sampling/Post Handling	Stage of Test Procedure		
		0 Days	28 Days	50 Days
Water				
Temperature (ºC)	--			
pH	5.7	8.049	5.640	4.440
Hardness (mg/L as $CaCO_3$)	39			
TOC (ppm)	5.6	4.7		3.3
O_2 Content (mg/L)	--	8.38	8.25	8.49
Redox potential (mV)		+314	+448	+430
Sediment				
Sampling Depth	--			
pH[1]	5.7	6.795	6.416	5.016
Soil Texture (USDA)	Sand			
Particle Size Distribution (%) — Sand	96			
Silt	3			
Clay	1			
Organic matter (%) (Furnace Method)	1.1			
Organic carbon (%) (Walkley Black)	0.49			
CEC (meq/100 g)	6.8			
Microbial biomass (µg C/g sediment)	--	159.1		220.5
Redox potential (mV)		-135	-92	-76

Data obtained from p. 31; Table 1, pp. 38-39; and Table 4, p. 43, in the study report. USDA sediment texture confirmed using USDA-NRCS technical support tools.
1 Post-handling pH of sediment determined as 1:2, sediment:0.01M $CaCl_2$.
-- = not reported.

B. Study Design

1. Experimental Conditions: Table 5 summarizes the experimental conditions.

Table 5. Experimental Design.

Experimental Design	Goose River		Chula	
Duration of the test	50 days			
Water:				
Type and size of filter used	2-mm			
Amount of sediment and water per treatment:				
Water (mL)	265		265	
Sediment (g dry wt)	64.175		118.637	
Water:sediment ratio	*ca.* 3:1, v:v (8 cm of water over 2.5 cm of sediment)			
Application rates:				
Nominal	1.0 µg a.i./mL			
Actual	0.936-1.030 µg a.i./mL			
Number of replicates:				
Control, if used	Sterile controls were not used.			
Treated	Duplicate samples (two entire vessels) of each test system were collected at each sampling interval.			
Test apparatus:				
Type/material/volume	The test system consisted of glass bottles (500-mL volume) containing flooded sediment that were connected in series (i.e., corresponding replicate samples of a given sediment system were connected) to a continuous flow-through volatile trapping system. Air was introduced into the system via a dip tube positioned beneath the water surface. Samples were preincubated under study conditions for 10 or 19 days (Goose River and Chula, respectively) prior to treatment. The test system is illustrated in Figure 2, p. 53.			
Details of traps for CO_2 and organic volatile, if any	Humidified air was continually pulled (*ca.* 15 mL/minute) through a series of samples, then through one Supelco ORBO-32 absorbent tube and two tubes of 2N KOH. The trapping apparatus is illustrated in Figure 2, p. 53.			
If no traps were used, is the system closed?	Volatile traps were used.			
Identity and final concentration (based on water volume) of co-solvent	Acetonitrile, <0.1% by volume			
Test material application method:				
Volume of the test solution used/treatment	185-210 µL/sample			
Application method (*i.e.*, mixed/not mixed)	Applied just below the water surface using a Rainin or Gilson Microman pipette.			
Any indication of the test material adsorbing to the walls of the test apparatus?	None.			
Microbial biomass in untreated samples (µg C/g sediment)	Initial	Final	Initial	Final
Water	Not reported			
Sediment	467.0	574.3	159.1	220.5
Experimental conditions:				
Temperature	20 ± 2°C, range not reported			
Continuous darkness (yes/no)	Yes			
Other details (if any)	Biomass was determined in untreated water:sediment systems.			

Data obtained from pp. 16, 20-22, 31; Table 2, pp. 40-41; and Figure 2, p. 53, of the study report.

2. Sampling during Study Period: Table 6 summarizes sampling during the study period.

Table 6. Sampling during Study Period.

Parameter	Details
Sampling intervals (duration)	0, 4, 7, 14, 17, 21, 28, and 50 days.
Sampling method	Duplicate samples (two entire vessels) of each test system were collected at each sampling interval.
Method of collection of CO_2 and organic volatile compounds	The KOH volatile traps were collected and replenished at each sampling interval. The ORBO-32 tubes were collected at study termination.
Sampling Intervals/Times	
Redox potential in water layer	Measured at each sampling interval.
Dissolved oxygen in water layer	
pH in water layer	
Redox potential in sediment	
pH in sediment	
Other details, if any	The water and sediment were separated on the day of sampling. The water was typically analyzed by LSC and HPLC within 24 hours of sampling. The sediment was extracted on the day of sampling and aliquots of the extract analyzed using LSC. Sediment extracts were stored frozen (*ca*. -20°C) prior to HPLC analysis, generally within 7 days. Water and soil extracts were stored frozen (-20°C) when not in use.

Data obtained from pp. 22-23, 35; Table 2, p. 41; and Table 4, p. 43, of the study report.

3. Analytical Procedures:

Separation of the Water and Sediment: The water layer was decanted from the sediment into Nalgene containers and centrifuged (p. 23; Figure 3, p. 54). Aliquots of the water were analyzed using LSC and HPLC.

Extraction/Clean Up/Concentration Methods: The sediment was mixed with acetonitrile, then transferred to Nalgene bottles used to centrifuge the water (p. 24, Figure 3, p. 54). Samples were extracted three times with acetonitrile by shaking at room temperature (60 minutes/extraction). After each extraction, the mixture was centrifuged and the supernatant decanted. Aliquots of the individual extracts were analyzed using LSC.

A portion of each sediment extract was filtered through a GF/C filter into an evaporation flask (p. 24, Figure 3, p. 54). The combined extracts were concentrated on a rocket evaporator, and the resulting residues were mixed with acetonitrile:water (50:50, v:v), then centrifuged. Aliquots were analyzed using LSC and HPLC.

Determination of Unextracted Residues: The extracted sediments were homogenized and analyzed for total radioactivity by LSC following combustion (p. 24; Figure 3, p. 54).

Determination of Volatile Residues: The KOH trapping solutions were analyzed using LSC (pp. 24-25). The presence of CO_2 in the KOH solutions was confirmed. The absorbent tubes were extracted with acetonitrile, and the extract was analyzed using LSC.

Total Radioactivity Measurement: Total [^{14}C] residues were determined by summing the concentrations of residues measured in the water, sediment extracts, extracted sediment, and trapping materials (Tables 5-6, pp. 44-47).

Derivatization Method: A derivatization method was not described.

Identification and Quantification of Parent and Transformation Compounds: Aliquots of the water and the sediment extracts were analyzed by HPLC using a Zorbax Eclipse XDB-C18 analytical column eluted with a gradient mobile phase of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile (pp. 26-27). The eluate was monitored with radioactivity and UV detectors. Chromatographic peak retention times were compared to those of reference standards.

To confirm the presence of trapped $^{14}CO_2$ as carbonate, the water and sediment extracts were analyzed by HPLC using a Hamilton PRP-C-18 column eluted with (A) 0.1% formic acid in water and (B) methanol (p. 27). The eluate was monitored with radioactivity and UV detectors.

To confirm the identification of isolated compounds, select fractions of the HPLC eluate were analyzed using LC-MS/MS (pp. 27-28).

Detection Limits (LOD, LOQ) for the Parent and Transformation Products: LSC Limits of Detection (LOD) were 0.002-0.006% of the applied for the KOH trap, 0.010-0.026% for the water layer, 0.012-0.032% for the sediment organic extracts, and 0.006-0.032% for soil combustion (Table 3, p. 42). The LSC Limits of Quantification (LOQ) were 0.008-0.021% for the KOH traps, 0.037-0.100% for the water layer, 0.045-0.122% for the sediment organic extracts, and 0.023-0.116% for soil combustion. HPLC LOD were 0.089-0.241% and 0.026-0.071% for the aqueous and organic solutions, respectively, and LOQ were 0.267-0.722% and 0.079-0.212%, respectively.

II. Results and Discussion

A. Data:

Study results, including total mass balances and distribution of radioactivity, are presented in **Tables 7a-7b**. In the water column of the Goose River water:clay loam sediment systems following treatment, measured redox potentials, oxygen saturation and pH were +260 to +393 mV, 7.50-8.53 mg/L and 8.155-8.651, respectively, with measured redox potentials and pH in the sediment of -148 to -81 mV and 6.449-7.266, respectively (Table 4, p. 43). In the water column of the Chula water:sand sediment systems following treatment, measured redox potentials, oxygen saturation and pH were +212 to +448 mV, 4.69-8.49 mg/L and 4.440-8.267, respectively, with measured redox potentials and pH in the sediment of -159 to -76 mV and 5.016-7.560, respectively. The pH of the water and sediment decreased steadily beginning at 14-17 days. In both test systems, the water was oxic and the sediment was suboxic during the experiment.

Microbial biomass in untreated Goose River clay loam sediment was 467.0 μg C/g sediment at time 0 and 574.3 μg C/g sediment at study termination (p. 31). Microbial biomass in untreated Chula sand sediment was 159.1 μg C/g sediment at time 0 and 220.5 μg C/g sediment at study termination.

B. Mass Balance:

In the Goose River water:clay loam sediment system, overall recoveries averaged 94.9 ± 6.9% (sample range 82.5-102.2%) of the applied (Table 5, pp. 44-45). Recoveries generally were near or below guideline criteria (<90%) beginning at 17 days. In the water columns, 2,4-DB was a maximum of 95.1% of the applied at time 0 and decreased to ≤0.1% at and after 17 days posttreatment (Table 7, p. 48). In the sediment, 2,4-DB was a maximum of 21.1% at 14 days and decreased to 2.1% at 50 days.

In the Chula water:sand sediment system, overall recoveries averaged 98.6 ± 5.2% (sample range 92.2-107.0%) of the applied (Table 6, pp. 46-47). Recoveries were within guideline criteria (90-110%). In the water columns, 2,4-DB was a maximum of 97.4% of the applied at time 0, decreased to 2.0% at 28 days posttreatment and was 0.0% at 50 days (Table 8, p. 50). In the sediment, 2,4-DB was a maximum of 13.0% at 7 days and decreased to 2.2% at 50 days.

C. Bound and Extractable Residues:

In the Goose River water, total radioactive residues decreased from 97.3% of the applied at time 0 to 1.6% at 50 days posttreatment (Table 5, pp. 44-45). In the sediment, extractable radioactivity increased from 4.4% at time 0 to a maximum of 26.3% at 14 days and was 4.2% at 50 days. Unextracted radioactivity increased from 0.8% at time 0 to a maximum of 28.9% at 28 days and was 18.7% at 50 days.

In the Chula water, total radioactive residues decreased from 98.7% of the applied at time 0 to 2.9% at 50 days posttreatment (Table 6, pp. 46-47). In the sediment, extractable radioactivity increased from 4.8% at time 0 to a maximum of 15.1% at 14 days and was 4.8% at 50 days. Unextracted radioactivity increased from 1.8% at time 0 to a maximum of 21.1% at 21 days and was 15.2% at 50 days.

D. Volatilization:

In the Goose River water:clay loam sediment systems, CO_2 was a maximum of 77.1% of the applied at 50 days posttreatment (Table 5, p. 44). Organic volatiles were <LOD at study termination.

In the Chula water:sand sediment systems, CO_2 was a maximum of 71.0% of the applied at 50 days posttreatment (Table 6, p. 46). Organic volatiles were <LOD at study termination.

Table 7a. Aerobic transformation of [^{14}C]2,4-DB, expressed as a percentage of the applied radioactivity, in Goose River water:clay loam sediment.

Sampling Interval (days)	0		4		7		14		17		21		28		50	
Replicate	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
2,4-DB	99.2	99.0	84.7	85.9	76.4	79.9	36.8	63.2	14.8	16.3	13.6	10.5	6.4	6.6	2.0	2.1
2,4-D	1.9	2.2	2.9	3.3	6.4	4.7	5.0	7.8	3.3	2.9	2.9	2.4	2.6	3.3	0.7	0.6
Carbonate	0.0	0.0	0.0	0.0	0.0	0.0	6.3	0.8	4.8	9.0	0.0	0.0	0.0	0.0	0.0	0.0
Others[1]	0.2	0.2	3.9	4.5	7.4	7.7	23.1	13.2	7.2	7.6	8.9	5.0	3.8	2.6	2.1	3.1
Water	96.8	97.3	75.0	76.1	68.6	69.7	47.3	58.6	11.4	15.7	7.8	3.6	3.5	2.4	1.0	1.6
Extracted residues	4.4	4.2	16.5	17.6	21.6	22.6	23.9	26.3	18.7	20.0	17.6	14.4	9.3	10.2	3.9	4.2
Unextracted residues	0.8	0.7	7.6	7.5	9.4	8.9	17.2	13.4	24.9	21.8	19.6	20.9	28.9	19.4	16.5	18.7
CO_2[2]	n.a.	n.a.	0.3	0.3	0.5	0.5	2.8	2.8	33.5	33.5	46.6	45.9	48.2	57.9	61.1	77.1
Organic Volatiles	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	<LOD	<LOD
Mass balance	102.1	102.2	99.3	101.4	100.1	101.8	91.2	101.1	88.5	91.0	91.6	84.7	89.9	89.9	82.5	101.6

Data obtained from Table 5, pp. 44-45, and Table 7, pp. 48-49, of the study report.
1 No single unassigned peak in the water or sediment was >4.1% of the applied (Table 7, p. 49).
CO_2 concentrations are averaged values (both replicates on a single trapping chain) through 17 days.
n.a. = not analyzed.

Table 7b. Aerobic transformation of [^{14}C]2,4-DB, expressed as a percentage of the applied radioactivity, in Chula water:sand sediment.

Sampling Interval (days)	0		4		7		14		17		21		28		50	
Replicate	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
2,4-DB	102.2	99.9	94.5	90.9	89.7	83.4	60.2	62.5	8.9	11.3	8.7	7.7	8.4	3.2	2.2	2.2
2,4-D	1.3	2.9	2.0	3.7	2.2	5.0	13.2	14.3	1.7	0.9	3.6	1.0	2.7	3.8	1.0	1.1
Carbonate	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.6	7.2	0.0	0.0	0.0	0.0	0.0	0.0
Others[1]	0.0	0.0	2.2	2.0	3.5	5.8	11.5	10.7	7.2	9.2	1.8	3.3	7.3	1.8	3.4	4.4
Water	98.7	98.2	86.3	83.9	81.8	79.9	71.1	72.3	15.6	16.0	5.9	4.6	9.3	3.6	1.8	2.9
Extracted residues	4.8	4.6	12.5	12.6	13.6	14.3	13.8	15.1	11.7	12.6	8.1	7.5	9.1	5.1	4.8	4.8
Unextracted residues	1.4	1.8	4.1	4.5	4.9	7.0	20.0	11.2	17.3	19.2	19.2	21.1	19.4	18.7	15.2	15.2
CO_2	n.a.	n.a.	0.7	1.3	0.6	1.0	2.1	3.0	50.7	44.7	63.8	59.1	57.1	64.7	71.0	71.0
Organic Volatiles	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	<LOD	<LOD
Mass balance	104.9	104.6	103.5	102.2	100.9	102.2	107.0	101.6	95.4	92.6	97.0	92.4	94.9	92.2	92.8	94.0

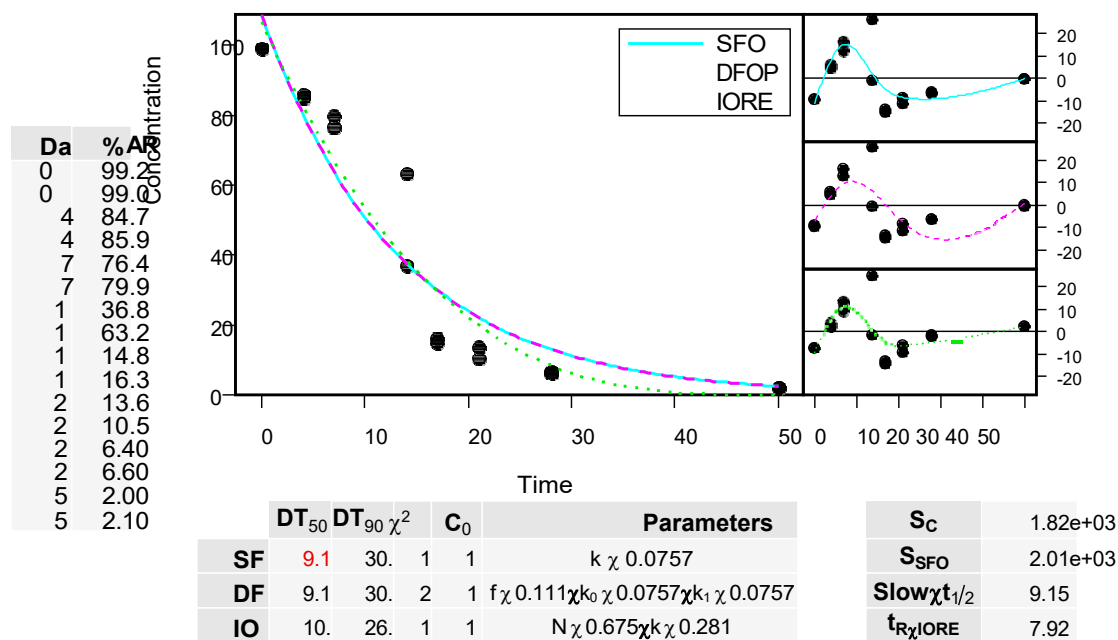Data obtained from Table 6, pp. 46-47, and Table 8, pp. 50-51, of the study report.
1 No single unassigned peak in the water or sediment was >5.7% of the applied (Table 8, p. 51).
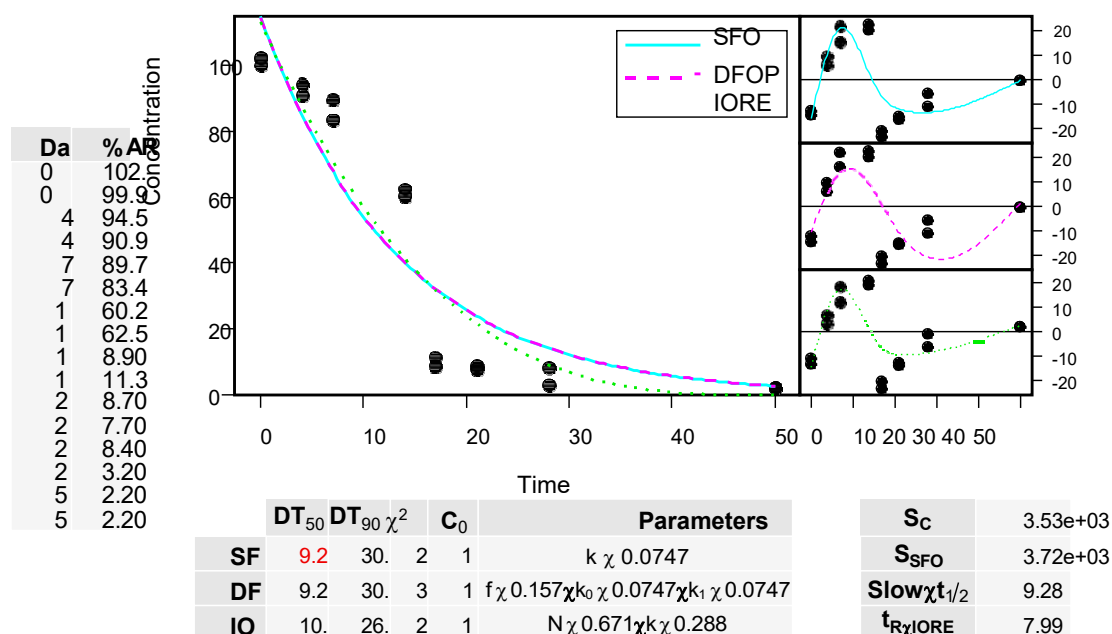n.a. = not analyzed.

E. Transformation of Parent Compound: Transformation kinetics of the parent compound in the total systems are summarized in the following **Figures.** Transformation product information is summarized in **Table 8**.

Using Single First Order (SFO) and Double First Order in Parallel (DFOP) models (<u>C</u>omputer <u>A</u>ssisted <u>K</u>inetic <u>E</u>valuation; CAKE v. 2.0), the study author determined total system SFO DT50 values of 8.98 days in the Goose River water:clay loam sediment system and 9.04 days in the Chula water:sand sediment system (pp. 29, 35; Figure 11, p. 69; Figure 13, p. 73).

2,4-DB in aerobic water:clay loam sediment

Da	%AR
0	99.2
0	99.0
4	84.7
4	85.9
7	76.4
7	79.9
1	36.8
1	63.2
1	14.8
1	16.3
2	13.6
2	10.5
2	6.40
2	6.60
5	2.00
5	2.10

	DT_{50}	DT_{90}	χ^2	C_0	Parameters		
SF	9.1	30.	1	1	$k \chi 0.0757$		
DF	9.1	30.	2	1	$f \chi 0.111 \chi k_0 \chi 0.0757 \chi k_1 \chi 0.0757$		
IO	10.	26.	1	1	$N \chi 0.675 \chi k \chi 0.281$		

S_C	1.82e+03
S_{SFO}	2.01e+03
Slow$\chi t_{1/2}$	9.15
$t_{R\chi IORE}$	7.92

2,4-DB in aerobic water:sand sediment

Da	%AR
0	102.
0	99.9
4	94.5
4	90.9
7	89.7
7	83.4
1	60.2
1	62.5
1	8.90
1	11.3
2	8.70
2	7.70
2	8.40
2	3.20
5	2.20
5	2.20

	DT_{50}	DT_{90}	χ^2	C_0	Parameters
SF	9.2	30.	2	1	k χ 0.0747
DF	9.2	30.	3	1	f χ 0.157 χk_0 χ 0.0747 χk_1 χ 0.0747
IO	10.	26.	2	1	N χ 0.671 χk χ 0.288

S_C	3.53e+03
S_{SFO}	3.72e+03
$Slow\chi t_{1/2}$	9.28
$t_{R\chi IORE}$	7.99

Kinetics models: Single First Order (SFO); Double First Order in Parallel (DFOP), and Indeterminate Order Rate Equation (IORE) in accordance with NAFTA kinetics guidance (USEPA, 2012).

Table 8. Transformation Products of 2,4-DB in the Aerobic Water:Sediment Systems.

	Transformation Products	Maximum %AR Observed	Associated Interval (days)	Final %AR Observed	Final Interval (days)
North Dakota USA Goose River water:clay loam sediment (20°C, water pH 8.0, sediment pH 7.5)	2,4-D	7.8	14	0.7	50
Georgia, USA Chula water: sand sediment (20°C, water pH 8.3, sediment pH 5.7)	2,4-D	14.3	14	1.1	50

Data obtained from Tables 7-8, pp. 48-51, in the study report.
Trapped CO_2 (as carbonate) was recovered from the water at a maximum 9.0-9.6% of the applied at 17 days from the two test systems.

A transformation pathway was provided by the study author (Figure 16, p. 77).
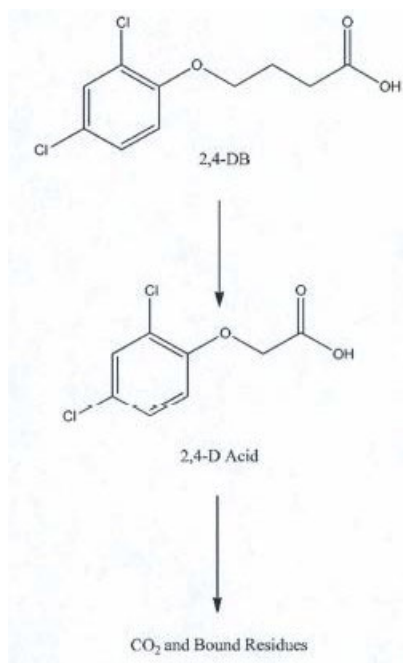
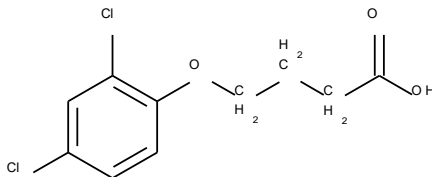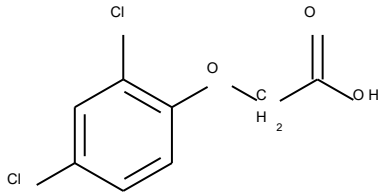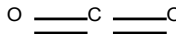## III. Study Deficiencies and Reviewer's Comments

1. The study author failed to use solvents with a range of dielectric constants to maximize extraction of the residues. Extractants included only acetonitrile and acetonitrile:water (50:50). Unextracted residues reached maximums of 21.1-28.9% of the applied (Tables 5-6, pp. 44-47). The study author stated that a preliminary experiment was conducted for 3 days to determine if the methods for extraction were adequate (pp. 31-32). It does not appear that additional solvents or any extraction procedure other than shaking at room temperature were tested. The likely change in the nature of the residues over time coupled with the possibility that different solvents would become more effective was not addressed.

2. In the Goose River system, there were high levels of unextracted residues present during the sampling intervals that comprise the time period in which the parent was detected at less than 50% of the applied. Inter-replicate variability was also significant during this same time period, with parent present at 36.8% and 63.2% of the applied at 14 days posttreatment, following a mean of 77.8% (7 days) and preceding a mean of 15.6% (17 days) present as parent. Unextracted residues were 21.8-24.9% of the applied at 17 days posttreatment. Additionally, mass balances had a greater than 10% decrease in 8 of 10 replicates from 14 to 50 days.

3. In the Chula system, there was a greater than 50% loss of parent between two consecutive intervals, with parent decreasing from a mean of 61.4% at 14 days to a mean of 10.1% by 17 days. At those two sampling intervals, unextracted residues were means of 15.6% and 18.3%, and mass balances in the four replicates ranged from 92.6-107.0% of the applied.
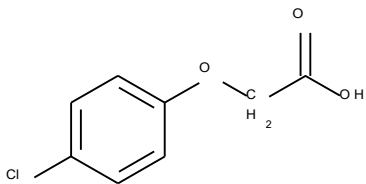
4. In the Goose River water:clay loam sediment system, overall recoveries were below guideline criteria (<90%) in multiple samples beginning at 17 days (Table 5, pp. 44-45). The study author attributed the loss of residues in the 50-day sample to loss of CO_2 (pp. 32-33).

5. In the Chula system, although overall recoveries did not drop below 90%, there were material balance losses of >10%, as the initial recoveries were a mean of 104.8%, and 5 of the 8 replicates in the last 4 sampling intervals were ≤94.0% of the applied.

6. It was not stated whether the reported redox potentials were measured or standard values. The probe used was not identified. The reviewer assumed that the reported values were measure, and used a default of +200 mV to convert from measured to standard values.

7. The study author stated that flasks were connected in series to the volatile trapping system, so that all samples for a given test system replicate shared the same set of traps (p. 20). Also, for the Goose River test system, both replicates were on the same trapping train through day 17 (Table 5, pp. 44-45). Therefore, the volatile concentrations that are reported at each interval are average values rather than sample-specific concentrations. It is preferred that sample vessels be connected to individual volatile traps so that more precise material balances for each sample can be calculated.

8. The pesticide use history at the test sites was not reported, and the water and sediments were not shown to be pesticide-free prior to use. The study author did state that fertilizers were not used at the site for the previous 4 years (p. 19).

IV. References

1. U.S. Environmental Protection Agency. 2008. Fate, Transport and Transformation Test - Guidelines, OPPTS 835.4300, Aerobic Aquatic Metabolism. Office of Prevention, Pesticides and Toxic Substances, Washington, DC. EPA 712-C-08-018.

2. U.S. Environmental Protection Agency. 2012. NAFTA Guidance for Evaluating and Calculating Degradation Kinetics in Environmental Media.

DER ATTACHMENT 1. 2,4-DB and Its Environmental Transformation Products. [A]

Code Name/ Synonym	Chemical Name	Chemical Structure	Study Type	MRID	Maximum %AR (day)		Final %AR (study length)
PARENT							
2,4-DB	**IUPAC:** 4-(2,4-Dichlorophenoxy)butyric acid **CAS:** 4-(2,4-Dichlorophenoxy)butanoic acid **CAS No.:** 94-82-6 **Formula:** $C_{10}H_{10}Cl_2O_3$ **MW:** 249.1 g/mol **SMILES:** OC(=O)CCCOc1ccc(Cl)cc1Cl		835.4300 Aerobic aquatic metabolism	49975801	PRT		PRT
MAJOR (>10%) TRANSFORMATION PRODUCTS							
2,4-D	**IUPAC:** (2,4-Dichlorophenoxy)acetic acid **CAS:** 2-(2,4-Dichlorophenoxy)acetic acid **CAS No.:** 94-75-7 **Formula:** $C_8H_6Cl_2O_3$ **MW:** 221.04 g/mol **SMILES:** O=C(O)COc(c(cc(c1)Cl)Cl)c1		835.4300 Aerobic aquatic metabolism	49975801	River water: clay loam	7.8% (14 d)	0.7% (50 d)
					Water:sand	**14.3%** (14 d)	1.1% (50 d)
Carbon dioxide	**IUPAC:** Carbon dioxide **CAS No.:** 124-38-9 **Formula:** CO_2 **MW:** 44 g/mol **SMILES:** C(=O)=O		835.4300 Aerobic aquatic metabolism	49975801	River water: clay loam	**77.1%** (50 d)	**77.1%** (50 d)
					Water:sand	**71.0%** (50 d)	**71.0%** (50 d)
Unextracted residues	NA	NA	835.4300 Aerobic aquatic metabolism	49975801	River water: clay loam	**28.9%** (28 d)	**18.7%** (50 d)
					Water:sand	**21.1%** (21 d)	**15.2%** (50 d)

Code Name/ Synonym	Chemical Name	Chemical Structure	Study Type	MRID	Maximum %AR (day)	Final %AR (study length)
**MINOR (<10%) TRANSFORMATION PRODUCTS**					
No minor transformation products were identified.					
**REFERENCE COMPOUNDS NOT IDENTIFIED**					
4-CPAA	**IUPAC:** (4-Chlorophenoxy)acetic acid **CAS:** 2-(4-Chlorophenoxy)acetic acid **CAS No.:** 122-88-3 **Formula:** $C_8H_7ClO_3$ **MW:** 186.6 g/mol **SMILES:** OC(=O)COc1ccc(Cl)cc1		835.4300 Aerobic aquatic metabolism	49975801	NA	NA

[A] AR means "applied radioactivity". MW means "molecular weight". PRT means "parent". NA means "not applicable".
Trapped CO_2 (as carbonate) was recovered from the water at a maximum 9.0-9.6% of the applied at 17 days from the two test systems.

Attachment 2: Statistics Spreadsheets and Graphs

Attachment 3: Calculations

Calculations were performed by the reviewer using R software, and the following equations.

Single First-Order (SFO) Model

$$C_t = C_0 e^{-kt} \tag{eq. 1}$$

where,

   C_t = concentration at time t (%)
   C_0 = initial concentration (%)
   e = Euler's number (-)
   k = SFO rate constant of decline (d^{-1})
   t = time (d)

The SFO equation is solved with R kinetics software by adjusting C_0 and k to minimize the objective function (S_{SFO}) shown in equation 9.

$$DT_{50} = \text{natural log (2)}/k \tag{eq. 2}$$

$$DT_{90} = \ln (10)/k \tag{eq. 3}$$

Indeterminate Order Rate Equation (IORE) Model

$$C_t = \left[C_0^{(1-N)} - (1-N)k_{IORE}t \right]^{\left(\frac{1}{1-N}\right)} \tag{eq. 4}$$

where,

   N = order of decline rate (-)
   k_{IORE} = IORE rate constant of decline (d^{-1})

This equation is solved with R kinetics software by adjusting C0, kIORE, and N to minimize the objective function for IORE (SIORE) (See equation 9). Half-lives for the IORE model are calculated using equation 5, which represents a first-order half-life that passes through the DT$_{90}$ of the IORE model. (Traditional DT$_{50}$ and DT$_{90}$ values for the IORE model can be calculated using equations 6 and 7.)

$$t_{IORE} = \frac{\log(2)}{\log(10)} \frac{C_0^{1-N}(1-0.1^{(1-N)})}{(1-N)k_{IORE}} \tag{eq. 5}$$

$$DT_{50} = \frac{(C_0/2)^{(1-N)} - C_0^{(1-N)}}{k(N-1)} \tag{eq. 6}$$

$$DT_{90} = \frac{(C_0/10)^{(1-N)} - C_0^{(1-N)}}{k(N-1)} \tag{eq. 7}$$

Double First-Order in Parallel (DFOP) Model

$$C_t = C_0 g^{-k_1 t} + C_0 (1 - g)^{-k_1 t} \tag{eq. 8}$$

where,

        g = the fraction of C_0 applied to compartment 1 (-)
        k_1 = rate constant for compartment 1 (d^{-1})
        k_2 = rate constant for compartment 2 (d^{-1})

If $C_0 \times g$ is set equal to a and $C_0(1\text{-}g)$ is set equal to c, then the equation can be solved with R kinetics software for a, c, k_1, and k_2 by minimizing the objective function (S_{DFOP}) as described in equation 9.

DT$_{50}$ and DT$_{90}$ values can be calculated using equations 2 and 3, with k_1 or k_2 in place of k.

Objective Function: SFO, IORE, and DFOP are solved by minimizing the objective function (S_{SFO}, S_{IORE}, or S_{DFOP}).

$$S_{SFO}, S_{IORE}, \text{ or } S_{DFOP} = \sum (C_{model}, t - C_{d,t})^2 \tag{eq. 9}$$

where,

        S_{SFO}, S_{IORE}, or S_{DFOP} = objective function of kinetics model fit ($\%^2$)
        n = number of data points (-)
        $C_{model,t}$ = modeled value at time corresponding to $C_{d,t}$ (%)
        $C_{d,t}$ = experimental concentration at time t (%)

Critical Value to Determine Whether SFO is an Adequate Kinetics Model

If S_{SFO} is less than S_C, the SFO model is adequate to describe kinetics. If not, the faster of t_{IORE} or the DFOP DT$_{50}$ for compartment 2 should be used.

$$S_c = S_{IORE} \left(1 + \frac{p}{n-p} F(\alpha, p, n-p) \right) \tag{eq. 10}$$

where,

        S_c = the critical value that defines the confidence contours ($\%^2$)
        p = number of parameters (3 in this case)
        α = the confidence level (0.50 in this case)
        $F(\alpha, p, n\text{-}p)$ = F distribution with α level of confidence and degrees of freedom p and n-p